PB86-117017

Quantitative Cancer Risk Assessment Methodology Using Short-Term Genetic Bioassays: The Comparative Potency Method

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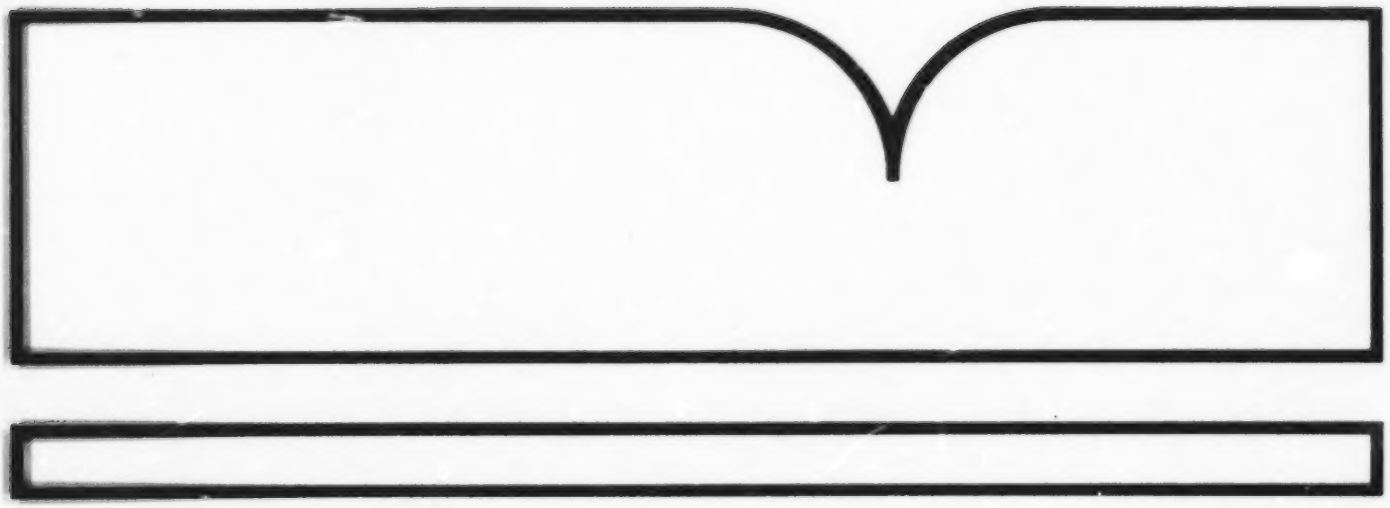
Research Triangle Park, NC

Oct 85



U.S. Department of Commerce
National Technical Information Service
NTIS

PB86-117017

EPA/600/D-85/237
October 1985

A Quantitative Cancer Risk Assessment Methodology Using Short-Term Genetic Bioassays:

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by

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SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA/600/D-85/237	2.	3. RECIPIENT'S ACCESSION NO. PB86 117017/AS
4. TITLE AND SUBTITLE A Quantitative Cancer Risk Assessment Methodology Using Short-Term Genetic Bioassays: The Comparative Potency Method		5. REPORT DATE October 1985 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Joellen Lewtas		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Environmental Protection Agency GBB/GTD/HERL MD68 Research Triangle Park, NC 27711		10. PROGRAM ELEMENT NO. A9RA1B 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA/600/11
15. SUPPLEMENTARY NOTES		

16. ABSTRACT

Quantitative risk assessment is fraught with many uncertainties. The validity of the assumptions underlying the methods employed are often difficult to test or validate. Cancer risk assessment has generally employed either human epidemiological data from relatively high occupational exposures or animal cancer data from lifetime rodent bioassays. Low-dose extrapolation methods are often used with the human epidemiological data to estimate risks at lower environmental exposure levels. Scaling factors are used with animal data to extrapolate from rodents to humans. The problems, controversies, and uncertainties associated with these methodologies has encouraged us to develop the concept and methodology associated with a comparative potency method for cancer risk assessment. This comparative potency method may be particularly appropriate for application to the quantitative cancer risk assessment of combustion emissions. Combustion sources emit soot particles containing carcinogenic polycyclic organic compounds which are mutagenic in short-term genetic bioassays in microbial and mammalian cells and are tumorigenic in animals. Although soot is considered to be a human carcinogen, soots from different combustion sources and fuels contain variable quantities of organic tars of differing compositions. This paper describes a comparative approach to evaluating such complex mixtures by utilizing short-term genetic bioassays. The methodology and assumptions inherent in the method are presented and discussed.

17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field Group
18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 16
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE $9.95

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

A QUANTITATIVE CANCER RISK ASSESSMENT METHODOLOGY USING SHORT-TERM GENETIC BIOASSAYS: THE COMPARATIVE POTENCY METHOD

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INTRODUCTION

Quantitative risk assessment is fraught with many uncertainties. The validity of the assumptions underlying the methods employed are often difficult to test or validate. Cancer risk assessment has generally employed either human epidemiological data from relatively high occupational exposures or animal cancer data from lifetime rodent bioassays. Low-dose extrapolation methods are often used with the human epidemiological data to estimate risks at lower environmental exposure levels. Scaling factors are used with animal data to extrapolate from rodents to humans. The problems, controversies, and uncertainties associated with these methodologies has encouraged us to develop the concept and methodology associated with a comparative potency method for cancer risk assessment. This comparative potency method may be particularly appropriate for application to the quantitative cancer risk assessment of combustion emissions.

Combustion sources emit soot particles containing carcinogenic polycyclic organic compounds which are mutagenic in short-term genetic bioassays in microbial and mammalian cells (Claxton and Huisingh, 1980; Lewtas, 1982; Holmberg and Ahlborg, 1983) and are tumorigenic in animals (Nesnow et al, 1982a,b). Although soot is considered to be a human carcinogen (IARC, 1985), soots from different conbustion sources and fuels contain variable quantities of organic tars of differing compositions. This presents a serious problem since it is not possible to collect human cancer

risk data for all the various possible exposures to combustion emissions. Furthermore is is impossible to collect human cancer data for exposures to emissions which have previously not been in the environment. The use of chronic animal testing for each new combustion technology developed would be prohibitively expensive and time consuming. This problem has motivated us to develop and test a comparative potency risk assessment methodology which would utilize short-term mutagenesis and tumorigenesis data.

A comparative data base was developed for the U. S. Environmental Protection Agency's Diesel Research Program (Lewtas et al, 1981; Nesnow and Lewtas, 1981). The comparative risk assessment methodology was subsequently developed using this data set (Albert et al, 1983). Although this method was initially developed to compare alternative combustion sources (e.g., engines) which are powered by different fuels (e.g., diesel vs. gasoline), this risk assessment model and methodology is applicable to the evaluation of various complex mixtures. This paper describes a comparative approach to evaluating such complex mixtures by utilizing short-term genetic bioassays. The methodology and assumptions inherent in the method are presented and discussed.

COMPARATIVE POTENCY METHOD

The comparative potency method for cancer risk assessment is based on the hypothesis that there is a constant relative potency between two different carcinogens across different bioassay systems. The mathematical expression for the constant relative potency model is the following:

$$\text{Relative Potency in Bioassay}_{(1)} = (k)\ \text{Relative Potency in Bioassay}_{(2)}$$

This assumption is implicit in any comparison which utilizes the relative toxicity of two substances in animals to determine which substance would most likely be less toxic to man. This constant relative potency assumption is a testable hypothesis, if the relative potency of two carcinogens in humans can be determined and compared to the relative potency in a series of in vitro and in vivo bioassays. The test of this model is whether there is a constant relationship (k) between the relative potencies in the two bioassays being compared such that:

$$\frac{\text{Relative Potency in Bioassay}_{(1)}}{\text{Relative Potency in Bioassay}_{(2)}} = \text{constant (k)}$$

We have tested this hypothesis for three complex organic emissions from a coke oven, roofing tar pot, and cigarettes by using human lung cancer data from epidemiological studies of humans exposed to these emissions (Albert et al, 1983). The relative potencies for coke oven compared to roofing tar were similar and ranged from 0.2-1.4 across data from humans, mouse skin tumor initiation, and selected short-term genetic bioassays as shown in Table 1. When the relative potency for cigarette smoke was compared to roofing tar or coke ovens, however, the short-term bioassays showed a much higher relative potency than the human and mouse skin tumor initiation bioassay (Table 1).

TABLE 1. Comparison of Relative Potencies of Emission Extracts in Humans and Bioassay Systems[a]

Bioassay	Roofing Tar / Coke Oven	Cigarette / Coke Oven	Cigarette / Roofing Tar
Human Lung Cancer	0.39	0.0024	0.0062
Tumor Initiation	0.20	0.0011	0.0055
Mouse Lymphoma (+MA)	1.4	0.066	0.047
Ames TA 98 (+MA)	0.78	0.52	0.67

[a] The relative bioassay potencies were determined for each of the bioassay systems by dividing the absolute activity (e.g., lung cancer risk, tumor initiation activiity or mutagenicity) of one emission by the activity of the other emission within that bioassay (Albert et al, 1983). The mouse skin tumor initiation data (Nesnow et al, 1982a; Nesnow et al, 1982b), mouse lymphoma data (Mitchell et al, 1981), and Ames data (Claxton, 1981) are reported and evaluated elsewhere (Lewtas, 1982; Albert et al, 1983). The collection and preparation of emission extracts have been previously reported (Lewtas et al, 1981).

A series of diesel and gasoline automotive emissions, for which no human data were available, was also tested in the battery of in vivo and in vitro bioassays. The relative potency within the automotive samples across all these bioassays was, however, relatively constant as shown in Table 2. High correlations (r^2 = 0.95-0.72) were observed between the mutagenic activity of five automotive emissions (diesel and gasoline) and their tumor initiating activity (Lewtas, 1982). The best correlations with tumor initiating activity were observed with the mouse lymphoma bioassay (r^2 = 0.95) and the Ames, TA98, -MA bioassay (r^2 = 0.90).

TABLE 2. Comparison of Relative Potencies of Diesel and Gasoline Emission Extracts in Several Bioassay Systems[a]

Bioassay	MA[b]	Auto 2 / Auto 1	Auto 3 / Auto 1	Auto 4 / Auto 1
Tumor Initiation		0.41	0.53	0.29
Mutation in Mouse Lymphoma Cells	-	0.23	0.29	0.90
	+	0.25	0.45	0.38
SCE in CHO Cells	-	0.25	----[c]	0.25
	+	0.42	0.24	----[c]
Mutation in Ames TA98	-	0.34	0.19	0.26
	+	0.23	0.11	0.26

[a]The relative potencies were determined by dividing the tumorigenicity or mutagenicity (linear slope of the dose-response) of one auto emission by the activity of the second auto emission within that bioassay. The data for each assay are reported elsewhere (Lewtas, 1982; Nesnow et al, 1982a; Albert et al, 1983; Claxton, 1981; Mitchell et al, 1981; Nesnow et al, 1982b). The generation, collection, and preparation of these automotive emissions have also been previously reported (Lewtas, 1982; Lewtas et al, 1981).

[b]MA = metabolic activation from rat liver S9.

[c]Insufficient data was available to calculate this relative potency

This method was initially applied to the estimation of a human life-time cancer risk (unit risk) for a series of diesel automobiles by comparison to the human cancer unit risk from coke oven, roofing tar, and cigarette according to the following equation (e.g., for coke oven emissions):

$$\text{relative human risk}\left(\frac{\text{diesel auto}}{\text{coke oven}}\right) = (k)\ \text{relative bioassay potency}\left(\frac{\text{diesel auto}}{\text{coke oven}}\right).$$

The relative potency in the mouse skin tumor initiation bioassay was directly used to estimate the cancer risk of diesel emissions. This approach to applying the comparative potency method assumes that when comparing between one of the three human carcinogens and the automotive emissions, the mouse skin is directly proportional ($k = 1$) to the relative potency in to humans. This assumption is supported by the correlation ($r^2 = 0.96$) between the relative potencies in mouse skin tumor initiating activity with the human lung cancer unit risks.

Since the relative mutagenic potency of the automotive emissions correlates so well with the relative tumorigenic activity, it is possible to calculate a proportionality constant to use in estimating the human risk to any one of these emissions from mutagenicity data as shown in Table 3. This calculation of k is also a test of the constant relative potency hypothesis. The relative potency constant ranged from 0.023 to 0.11 or approximately five-fold with the average $k = 0.05$.

The major assumption and main source of uncertainty in this comparative potency method is the validity of the constant relative potency hypothesis. Interspecies differences in absorption and metabolism, which present the most difficult problems in extrapolation from animals to humans, may also affect the relative carcinogenic potency. By developing and evaluating a comparative data base across human, animal, and short-term bioassays for the same substances, these assumptions can be tested. The constant relative potency model does not assume "a priori" that the carcinogens being compared are chemically similar. The coke oven, roofing tar, cigarette smoke, and automotive emission samples are similar in that they all contain polycyclic organic compounds which cause frameshift mutations in Salmonella typhimurium (Claxton and Huisingh, 1980). These four emissions differed in their metabolic

activation requirements (Claxton, 1981), and upon chemical fractionation and characterization significant differences were observed in the specific mutagenic/carcinogenic chemicals present (Lewtas, 1984). The similarity in chemical composition of the different diesel automobile emissions may however be the basis for the constant relative potency observed across the short-term bioassays and animal tumorigenicity for this series of emissions. The mutagens and carcinogens present in these mixtures may differ only in their concentration.

TABLE 3. Relative Potencies of Automotive Emissions Compared to Coke Oven

	Relative Potency		
Emission Sample	Mouse Skin Tumorigenesis[a]	Ames[b]	k[c]
Coke Oven Topside	1.0	1.0	-
Diesel Auto 1	0.28	12.0	0.023
Diesel Auto 2	0.11	2.7	0.041
Diesel Auto 3	0.15	1.4	0.11
Gasoline Auto 4	0.08	3.2	0.025
			Ave = 0.05

[a]Relative potency determined by dividing the tumorigenic potency of each emission by the tumorigenic potency of the coke oven topside sample.

[b]Relative potency determined by dividing the mutagenic potency in the Ames Salmonella typhimurium TA 98 bioassay in the presence of metabolic activation of each emission by the mutagenic potency of the coke oven topside sample.

[c]$k = \frac{\text{relative potency in mouse skin}}{\text{relative potency in Ames}}$

EXPERIMENTAL METHODS

Emission Sample Collection and Extraction Methods

Combustion emissions contain both gaseous and particulate matter. Only the particulate matter containing condensed organics were included in the samples described here with the exception of the cigarette smoke condensate sample. Particulate matter with the condensed organics (soot) from combustion emissions are typically collected by filtration or electrostatic precipitation after dilution and cooling (Bradow, 1982; Schuetzle, 1983). When directly collected by condensation (Grimmer et al, 1973) the sample may also contain some of the gaseous emissions. The automotive emissions described here as well as the residential oil and wood combustion emissions were collected by a modification of the standard dilution tunnel method (Lewtas et al, 1981; Bradow, 1982) where the exhaust is collected on large 20"x20" Teflon-coated Pallflex T60-A20 filters. The collection methods for the coke oven, roofing tar, and cigarette smoke condensate have been described elsewhere (Lewtas et al, 1981). The particulate samples were Soxhlet extracted with dichloromethane (DCM) for 48 hr, filtered (0.2 μm pore) and the DCM removed by evaporation and solvent-exchanged into dimethylsulfoxide (DMSO) for all the bioassays discussed here except for mouse skin tumorigenesis where acetone was used as the solvent.

Bioassay Methods

The short-term genetic bioassays in which dose-dependent and reproducible increases in response were obtained for many of the combustion emission samples evaluated included:

1. Reverse mutation in Salmonella typhimurium (Ames bioassay) (Claxton, 1981).

2. Forward mutation at the thymidine kinase locus in L5178Y mouse lymphoma cells (mouse lymphoma bioassay) (Mitchell et al, 1981).

3. Sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cells (SCE bioassay) (Mitchell et al, 1981).

For these bioassays, one or more preliminary range-finding tests were performed to determine the optimum dose range for defining the slope of the dose-response. The bioassays were generally performed with duplicate or triplicate treatments at five to seven doses. The bioassays were all performed in the presence and absence of an Aroclor-induced rat liver S9 metabolic activation system (MA). The linear slope of the dose-response determined from simple regression analysis was utilized for the comparative potency analysis as previously described (Albert et al, 1983). The fit to linear response was generally good and the r^2 for each linear regression analysis is reported elsewhere (Lewtas, 1982).

Mouse skin tumorigenesis assays which have had considerable use in determining the tumorigenic and carcinogenic effects of chemicals and complex mixtures, were performed in SENCAR mice as previously described (Nesnow et al, 1982a) utilizing five single doses (80 mice/dose) and scoring individually at six months for papillomas and at one year for squamous cell carcinomas (Nesnow et al, 1982b). The carcinoma responses in the initiation assay were correlated with the papilloma responses as described by Nesnow et al, 1982b; only the papilloma responses were used in the comparative potency analysis (Albert et al, 1983).

COMPARATIVE ASSESSMENT OF COMPLEX MIXTURES FROM ALTERNATIVE ENERGY SOURCES

Short-term bioassays have proven to be useful in evaluating the effects of various engines, fuels, and control technologies on the mutagenicity of emissions. To draw meaningful conclusions from such comparisons, however, the normal day-to-day variations in the emissions were studied for one engine under standard conditions (Claxton and Kohan, 1981). The coefficients of variation (CV) for the particulate emission rates, percent organic extractables, and mutagenic activity ranged from 0.07 to 0.11. To compare the mutagenicity of particulate emissions from different sources or fuels, the percent of organic material extractable from the particles and the vehicle's particulate emission rate must also be included in the analysis. The final rate of mutagenic emissions is determined from all three parameters.

Although pure compounds have been reported to range in carcinogenic and mutagenic potency over six orders of magnitude (Meselson and Russel, 1977), the relative potency of combustion emissions ranged from only a five-fold difference in the mouse skin tumorigenesis assay to a 150-fold difference in the mouse lymphoma gene mutation assay. The Ames Salmonella typhimurium mutagenesis assay showed approximately two orders of magnitude difference (100-110 fold) in mutagenic activity from the weakest to the most potent emission sample when measured per mass of extractable organics (e.g., rev/μg). The emission rates (rev/km or rev/J) for the particle-bound organics, however, differed in some cases by as much as five orders of magnitude (10^5). To directly compare the mutagenicity of emissions from various sources, it is critical to compare the rates of mutagenic emissions on the same basis (e.g., fuel consumption, distance driven, energy consumption, or yield). To compare rates of mutagenic emissions of mobile sources, we have compared the mutagenic activity per kilometer, as shown in Table 4. The rates of mutagenic emissions from stationary sources are compared on the basis of fuel or energy consumption, as shown in Table 5.

This comparative potency method provides a framework for evaluating alternative energy sources, fuels, or control technologies by comparing them to the conventional technologies in short-term genetic bioassays. It is possible to extend the method described here for estimating lung cancer unit risks for diesel and gasoline automotive emissions to unit risk estimates for various combustion emissions or other complex mixtures through the data base established linking human, animal, and short-term genetic bioassay data. A simplified comparative approach to evaluating alternative energy sources is to employ parallel bioassay studies of the alternative (a) and conventional (c) source emissions and determine a relative risk by direct comparison as follows:

increased risk (a/c) = relative bioassay potency (a/c).

Since there is no one conventional standard petroleum-derived fuel or one standard combustion source, such studies need to consider the range of mutagenic and carcinogenic potency between different conventional sources and fuels. The establishment of such a range could then serve as a guide for evaluating alternative fuels or sources. For

TABLE 4. Comparative Mutagenic Emission Rates for Transportation Combustion Sources

Vehicles[a]	Fuel type	Mutagenicity of Organics[b] Ames TA98 (+MA)	Mouse Lymphoma (+MA)	Organic Emission Rate (mg/km)	Mutagenic Emission Rates (activity/km)[c] Ames TA98 ($x10^5$)	Mouse Lymphoma ($x10^5$)
Diesel engine						
Car (Mercedes)	diesel	12.0	1.5	19.2	2.4	0.30
Truck (Ford/Cat)	diesel	1.7	0.28	302.0	5.3	0.85
Bus (GM)	diesel	0.1	0.35	357.0	.37	1.3
Gasoline engine						
Non-catalyst (Ford Van)	unleaded gasoline	32.0	5.7	5.7	1.8	0.32
Catalyst (Mustang II)	leaded gasoline	3.5	1.1	1.4	.05	0.02

[a] The methods for collecting these emissions and performing the mutagenesis bioassays have been reported elsewhere (Lewtas, 1982; Lewtas et al, 1981; Lewtas, 1984).
[b] The mutagenicity of the organics in the Ames bioassay is expressed in revertants/µg (rev/µg) and in the mouse lymphoma bioassay as mutants/10^6 survivor/µg/ml (mut. freq./µg/ml).
[c] The mutagenic emission rates were determined from multiplying the mutagenicity of the organics times the organic emission rate.

TABLE 5. Comparative Mutagenic Emission Rates for Stationary Energy Combustion Sources

Residential[a]	Fuel type	Mutagenicity of Organics Ames TA98[a,b] (rev/μg)	Organic Emission Rate (ng/J)	Mutagenic Emission Rate[c] (rev/J) ($x10^3$)
Woodstove (airtight)	Pine	1.3	508.0	660.0
Woodstove (airtight)	Oak	0.9	187.0	168.0
Oil furnace #1	Oil #2	2.0	0.5	1.0
Oil furnace #2	Oil #2	5.1	1.5	7.6
Utility power plants				
Conventional	coal	3.1	0.01	0.031
Fluidized-bed	coal	9.4	0.03	0.28

[a] The methods for collecting these emissions and performing the mutagenesis assays have been reviewed elsewhere (Lewtas, 1984).
[b] The mutagenicity of the woodstove and oil furnace emissions is shown in the presence of metabolic activation (MA) and the utility coal power plant emissions in the absence of MA.
[c] The mutagenic emission rates were determined by multiplying the mutagenicity of the organics times the organic emission rate.

example, if the relative potency of the alternative source or fuel emissions fell within the range for the conventional fuels, one could assume no increased human risk. The battery of bioassays to be employed in such a study would depend upon availability of emission sample, resources, and time. As more bioassays are employed, however, confidence in the relative potency estimates should increase.

This direct comparative analysis of mutagenic emission rates provides a simple means of evaluating alternative energy sources. It is apparent from this data base that the organic emission rate (e.g., rev/kg fuel) may vary over over a wider range than the mutagenicity or carcinogenicity of the emissions (e.g., rev/mg particles). Therefore, when directly comparing sources the mutagenic or tumorigenic emission rate should be compared.

The increased or decreased human cancer risk from combustion emissions may be of greater concern than the absolute risk from these emissions when we consider the effect of alternative energy technologies and fuels. The data of greatest value in determining this increased or decreased risk is comparative bioassay data on emissions from the complex mixture being evaluated or comparative bioassay data on the conventional and alternative technology being evaluated. Such studies should be useful in providing direction for engineers and chemists to design alternative energy sources and fuels which result in less mutagenic and potentially less carcinogenic emissions.

The comparative potency method for cancer risk assessment described in this paper cannot be employed without uncertainty or without invoking the constant relative potency assumption inherent in the method. It is important to recognize that the assumptions and uncertainties are different than those employed in either using low-dose extrapolation techniques with human data or scaling factors with animal data. This makes it possible, therefore to perform quantitative cancer risk assessments using more than one approach with different methodological assumptions. In the future, more information may be gained by comparing the quantitative assessments for one source using several independent cancer risk assessment methodologies.

DISCLAIMER

This paper has been reviewed by the Office of Research and Development, U.S. Environmental Protection Agency and has been approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency.

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